

Original article

Seasonal trends in house dust mite allergen in children's beds over a 7-year period

Background: House dust mite (HDM) allergy is closely linked to the expression of asthma and other allergic diseases. Understanding factors influencing variation in allergen may help in controlling allergic disease. The objective of this study was to investigate the effects of seasonal changes in climate, type of bed used in very early childhood and anti-mite interventions on HDM allergen concentration.

Methods: Participants were enrolled in a randomized-controlled trial of HDM avoidance. Der p 1 was measured in dust samples from children's beds on 13 occasions, from birth to age 5 years, between 1997 and 2004. Bed types were categorized as bassinette, cot or bed. The effects of study month, type of bed and intervention group on HDM allergen concentration were estimated by multiple linear regression. The relation between climatic variables and HDM allergen concentration was investigated using a polynomial distributed lag model.

Results: House dust mite allergen concentrations were initially low in cots and bassinets in 1997/1998, peaked in bassinets and beds between 1999 and 2001 and then slowly declined during the period 2002/2004. Seasonal fluctuations occurred with minima in summer and two- to threefold higher maxima during late autumn. Allergen peaks were correlated with relative humidity peaks 2 months previously. Seasonal changes in allergen were not affected by the HDM avoidance intervention.

Conclusions: House dust mite allergen concentrations in Sydney beds fluctuate approximately two- to threefold on an annual cycle, partly determined by relative humidity, with peaks in late autumn and minima in summer. Fluctuations of this magnitude might be sufficient to influence asthma symptoms.

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Key words: allergens; Der p 1; house dust mite; intervention; season.

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Accepted for publication 23 July 2007

House dust mites (HDM), mainly comprising the species *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, have a wide global distribution, with larger populations occurring in temperate regions and smaller populations in more arid climates. Such mites commonly inhabit domestic fabrics in beds, furnishings and carpets (1). The concentration of the mite allergen Der p 1 in dust from these sites, expressed as µg allergen/g dust, is widely used as a proxy for exposure, which is linked to the occurrence of mite sensitization and allergic diseases, particularly asthma (2–4). The role of environmental factors determining mite allergen concentrations has been extensively studied as it may constitute risk factors for asthma exacerbations and indicate opportunities for interventions.

Abbreviations: BOM, Australian Bureau of Meteorology; CAPS, Childhood Asthma Prevention Study; ELISA, enzyme-linked immunosorbent assay; HDM, house dust mite; RH%, per cent relative humidity.

Our understanding of the potential impact of climate on mite allergen concentrations is mainly based on studies of culturing mites at different combinations of temperature and humidity (5, 6). Seasonal fluctuation of mites or their allergens in houses have been observed in several studies, although the timing of peaks may occur in different seasons depending on the locality. Peaks in mite numbers have been reported during autumn (7–9), winter (10), spring (11) and summer (12–16), while peaks in allergens have been reported in autumn (17–22), autumn–winter (23), summer (16, 24) and summer–autumn (7, 25). Some studies found no fluctuations (26–29). However, many of these only sampled for a year or less and none sampled for more than 2 years. Furthermore, no studies have reported whether allergen avoidance interventions in beds affect the seasonal variation in allergen concentrations.

House dust mite allergen concentrations in Sydney, Australia are relatively high compared to other countries. The climate is temperate with year-round rainfall. Two

previous small studies in Sydney showed inconclusive results. One showed autumn peaks in carpets but not beds (30), whereas another showed autumn peaks in beds (31).

The Childhood Asthma Prevention Study (CAPS) is a randomized-controlled trial of HDM avoidance, implemented from birth to age 5 years. Samples of bed dust were regularly collected over a total period of 7 years as the cohort progressed through early childhood. CAPS represents an excellent opportunity to study both the relationship between macroclimate fluctuation and the concentration of mite allergen in the different types of beds used and whether these patterns are modified by an intervention.

Methods

Subjects and HDM allergen intervention

The design and main outcomes of CAPS has been reported previously (32, 33). The study antenatally recruited 616 infants born in Sydney between 1997 and 1999. The infants were randomized at birth into HDM avoidance or non-intervention groups. The intervention used allergen-impermeable mattress and pillow covers and washed all bedding every 3 months in an acaricidal detergent. Both groups received additional verbal advice about allergen control. During the course of the study children progressed from bassinets, to cots and then to beds at the discretion of their parents. A total of 516 subjects completed the study at age 5 years.

This study was approved by the Human Research Ethics Committees of the University of Sydney, Children's Hospital Westmead, and Western and South Western Sydney Area Health Services.

Sample collection and Der p 1 determination

Dust was collected on 13 occasions from the children's beds: at ages 1, 3, 6, 9 and 12 months and then at 6-monthly intervals, up to the age of 5 years. The total sampling period for the study was from the 10 November 1997 to the 24 January 2005 (7 years).

Samples comprised fine dust pre-filtered through a 150 µm nylon mesh, and collected in a 20 µm nylon mesh bag, using a 1000 W vacuum cleaner. The sample contained dust vacuumed from the upper bedding (e.g. duvet and blankets), the bedding immediately under the lower sheet, and the pillow(s). The above sites were vacuumed for 1 min, 30 s and 30 s, respectively.

As previously described (33), 50 mg of dust was extracted in 1 ml of phosphate-buffered saline solution (pH 7.4) containing 0.2% (w/v) bovine serum albumin and 0.05% (v/v) Tween-20 and the extracts stored at -20°C. The concentration of mite allergen Der p 1 was determined by double monoclonal enzyme-linked immunosorbent assay (34) using a commercially available kit (EL-DP1, Indoor Biotechnologies Ltd, Cardiff, UK). Der p 1 allergen measurements were expressed as a concentration of µg/g of fine dust.

Collection of data during home visits

Indoor and outdoor RH% and temperature were recorded at the time of dust collection using a digital thermohygrometer (Cole-Palmer Instruments Pty Ltd, Vernon Hills, IL, USA). Indoor readings were made in the subject's bedroom and outdoor readings in a shaded part of their garden. Information on the type of bed was also recorded.

Climate data

Regional data on climate (3 hourly air temperature and vapour pressure) for eight climate stations, representing the regions the subjects were recruited from, was obtained from the Australian Bureau of Meteorology (BOM) for the entire study period.

These data were used to calculate mean outdoor air temperature and RH% for each month of the study, as an 85-month time series, and also for each calendar week of the year, averaged over the 7-year study period. For each month, average temperature was calculated as the mean of all temperature data collected for the month. Average RH% for each month was calculated from the average temperature and vapour pressure for the month using a modified version of Tetens formula (35). Average temperature and RH% for each month for the subjects' houses were derived in the same way.

Statistical analyses

Dust samples were excluded from the analysis whenever they weighed < 5 mg ($n = 195$); had a Der p 1 concentration below the detection limit of 2.8 ng/ml ($n = 8$); were collected from a bed the child was sharing with a parent or another child ($n = 561$); or were not described as coming from a bassinet, cot or bed ($n = 113$).

Der p 1 allergen measurements were log₁₀-transformed prior to analysis.

The effects of the type of bed, subject's age, month of sampling (0–85) and HDM intervention group (HDM avoidance vs non-intervention) on Der p 1 allergen concentrations were tested in a multiple linear regression model in which subjects were included as a random effect (PROC MIXED, SAS 9.01, SAS Institute, Cary, NC, USA). Three additional interaction variables were also tested as fixed effects. These variables examined whether monthly fluctuations in allergen concentrations differed between the two intervention groups, the three types of beds analysed and whether the intervention had varying effects on these different types of beds. Covariates that significantly predicted the outcome variable ($P < 0.05$) were retained in the final model. Least-squares mean Der p 1 concentrations for each month, adjusted for the significant covariates, were estimated and displayed graphically.

To examine the pattern of Der p 1 allergen concentrations occurring during the course of a year, a similar mixed model was developed using each week of the year (1–52) as a fixed effect, instead of the month of study.

The distribution and magnitude of the lagged effect of RH% on Der p 1 concentrations was modelled using a polynomial distributed lag model (PROC PDLREG, SAS 9.01, SAS Institute) and displayed graphically. We considered polynomials to the third degree with up to 12 lags, allowing monthly Der p 1 concentrations to be influenced by regional RH% over the previous 12 months.

Results

Of the 6833 bed dust samples collected over the study period, 5955 samples from 587 subjects in 1054 different houses were suitable for analysis; a mean of 69 samples (95% CI: 63–76) per month. There were 504 samples from bassinets, 2834 from cots and 2617 from beds, of which the HDM avoidance group comprised 48%, 49% and 49%, respectively. These samples exclude one outlier, with a Der p 1 concentration > 1 mg/ml. Data from the first and last month of the study were included in the

calculations, but omitted from the final plots of the time series, as their small sample size produced large confidence intervals.

Seasonal fluctuations in Der p 1 allergen concentrations

The overall median Der p 1 concentrations were 4.6 µg/g (IQR: 1.7–14.6) for the HDM avoidance group, and 16.6 µg/g (IQR: 5.8–34.6) for the non-intervention group. After adjustments for the type of bed, HDM intervention group and the interaction of these, the month of sampling was a significant predictor of HDM allergen concentration in beds ($P < 0.001$). Age of the subjects did not

influence Der p 1 allergen concentrations after adjusting for these factors ($P > 0.2$). When the differences in the distribution of bed types between the two intervention groups have been adjusted for, the overall geometric mean Der p 1 concentrations were 4.38 µg/g (95% CI: 3.91–4.90) in the HDM avoidance group and 9.77 µg/g (95% CI: 8.74–10.92) in the non-intervention group.

Der p 1 allergen concentrations in the subject's beds were low during 1997/1998, rose sharply and peaked between 1999 and 2001, and then slightly declined during 2002–2004 (Fig. 1, geometric mean Der p 1).

Yearly peaks of mite allergen generally occurred in late autumn (April and May in the southern hemisphere).

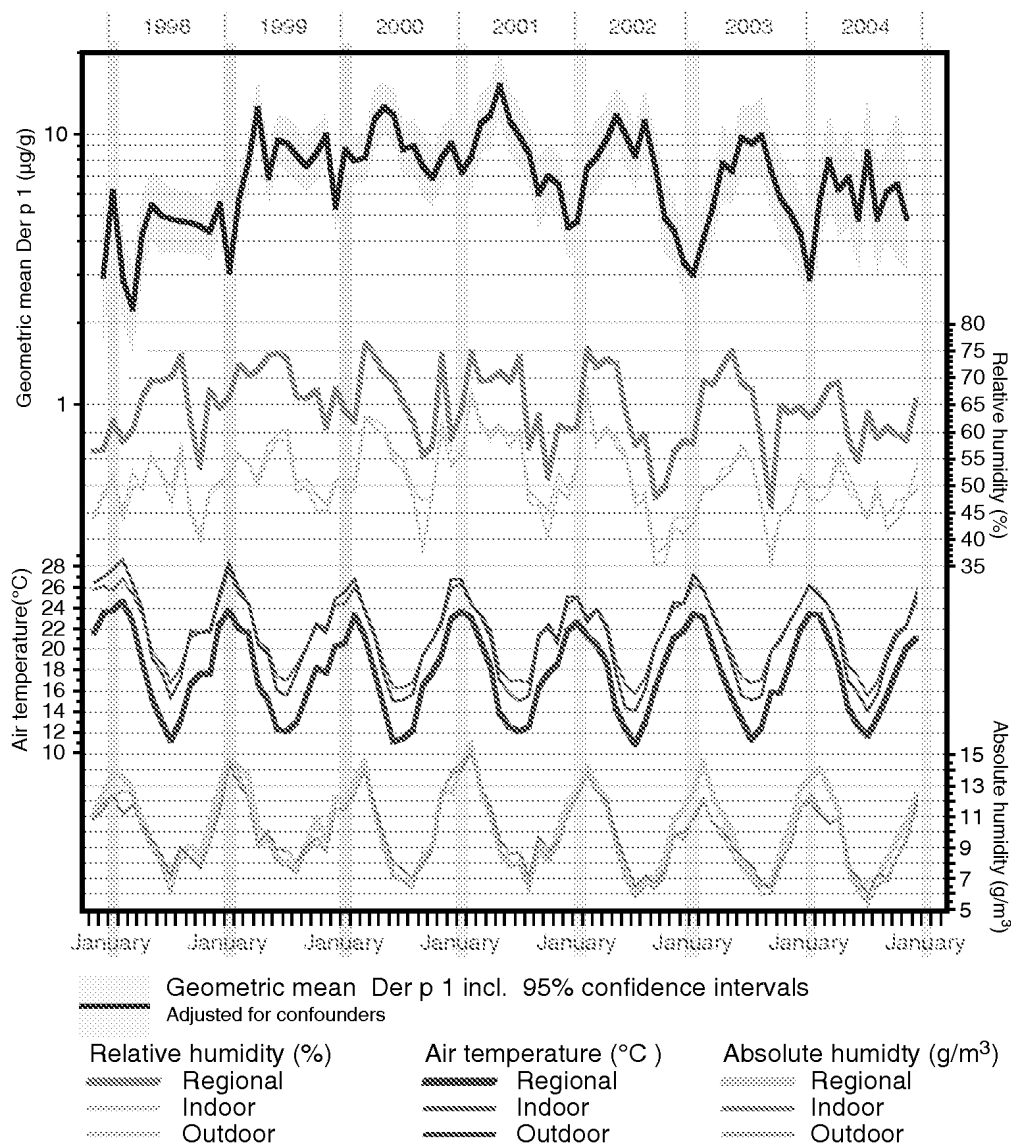


Figure 1. Time series plot of monthly geometric mean Der p 1 concentration in children's beds, adjusted for confounders (bed type, month of sampling, intervention group and the interaction of the intervention on the three types of bed analysed) with average monthly climate measures, from November 1997 to December 2004. Regional climate measures were obtained from the Australian Bureau of Meteorology. Indoor and outdoor climate measures were recorded at the site and time of sampling.

Three exceptions occurred: in 1998 there was an additional peak in December and later peaks in 2003 (August) and 2004 (July). The largest annual peak of 15.2 µg/g (95% CI: 11.7–19.8) occurred in May 2001, the lowest annual peak of 5.4 µg/g (95% CI: 4.1–7.3) occurred in May 1998.

Yearly minimums of mite allergen concentrations generally occurred in mid-summer (January in the Southern Hemisphere). There were two exceptions; in 1998 the minimum occurred in March and the minimum for 2000 occurred in December 1999. Yearly minimums ranged from 7.2 µg/g (95% CI: 5.5–9.4) in 2001 down to 2.3 µg/g (95% CI: 1.5–3.3) in March 1998. In the period 1999–2003, where seasonality was most evident, the average minimum was 37% (95% CI: 29–43) of the average maximum.

Climate fluctuations during the study

In Sydney, temperatures are highest in January and February and the lowest in June and July (Fig. 1, regional air temperature). The pattern of RH% showed less definite annual fluctuations than temperature. The highest RH% generally occurred from late summer to the end of autumn (between February and May). The maximum monthly peaks in RH% varied between 69.2% and 76.4%. Monthly lows in RH% generally occurred during spring (September or October; Fig. 1, regional relative humidity). Absolute humidity mainly peaked in February (Fig. 1, regional absolute humidity).

There was little difference between indoor and outdoor humidity measurements (Fig. 1, indoor and outdoor relative humidity), which are lower than the corresponding BOM data (Fig. 1, regional relative humidity). The outdoor air temperature measurements collected during house visits, were slightly higher than the regional data obtained from BOM, and the indoor temperatures

fluctuated less than the concurrent outdoor temperatures over a year (Fig. 1, air temperature). There was very little difference in monthly mean humidity, when this was calculated as absolute humidity (Fig. 1, absolute humidity).

On average, over the 7 years of the study, peaks occurred over 9 weeks in autumn and winter (weeks 17–25), and minima occurred during the first 9 weeks of summer (week 50 to week 6 of the following year). A notable feature was the stability of the allergen concentrations throughout spring (weeks 36–48; data not shown).

Effects of the intervention and different bed types on Der p 1 allergen concentration

The month-to-month variation in allergen concentration did not differ significantly between either the two intervention groups ($P = 0.10$) or between the three bed types examined ($P = 0.06$; Fig. 2). However, the HDM avoidance intervention had a greater effect in reducing HDM allergen concentrations in cots than in other bed types (Table 1, $P < 0.001$ for the interaction between type of bed and the intervention group).

Effect of relative humidity on HDM allergen

Der p 1 concentrations in the beds were significantly correlated with the RH% over the preceding 6 months but not during the month of sampling. The strongest correlation was with RH% 2 months earlier ($P < 0.001$; Fig. 3).

Discussion

This study provides the most comprehensive set of observations to date on seasonal fluctuations of mite allergens in children's beds with climate. In this temperate

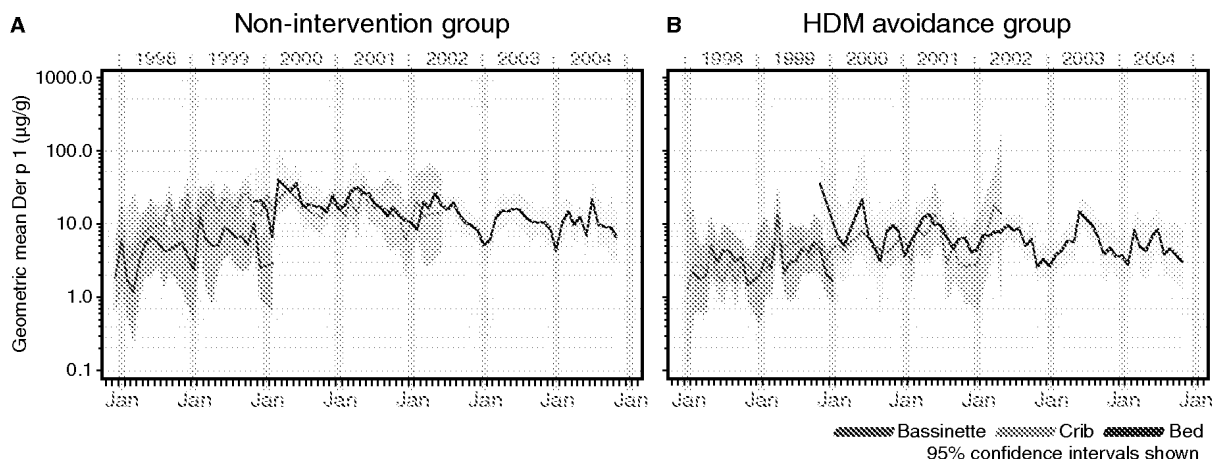


Figure 2. Time series plots of monthly geometric mean Der p 1 concentration in three bed types differentiated by colour, from the (A) non-intervention group and (B) the house dust mite avoidance group, from November 1997 to December 2004, without any adjustments. Monthly mean values using < 5 measurements were omitted from these plots.

Table 1. Effects of the house dust mite (HDM) intervention and the type of bed on HDM allergen concentration in beds

	Ratio (95% CI)
HDM avoidance group vs non-intervention group	
In beds	0.47 (0.40–0.55)*
In cots	0.28 (0.24–0.33)*
In bassinettes	0.68 (0.54–0.86)
Beds vs cots	
In HDM avoidance group	1.50 (1.32–1.69)*
In non-intervention group	0.91 (0.81–1.03)
Beds vs bassinettes	
In HDM avoidance group	1.74 (1.42–2.12)*
In non-intervention group	2.55 (2.09–3.10)*
Bassinettes vs cots	
In HDM avoidance group	0.86 (0.73–1.02)
In non-intervention group	0.36 (0.31–0.42)*

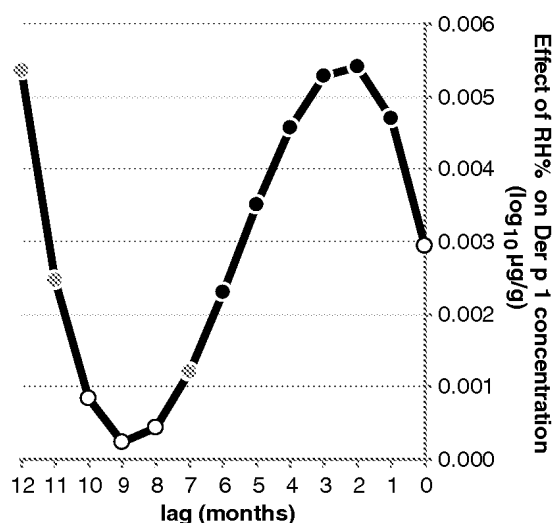
* $P < 0.001$.

Figure 3. Plot of the estimated lag distribution as modelled by the polynomial distributed lag model, showing the effect of RH% on the concentration of Der p 1 in beds ($\log_{10} \mu\text{g/g}$), with Der p 1 concentration being influenced by RH% for up to 12 months preceding the date of sample collection (lag). Highly significant ($P < 0.001$) effects are shown as black circles, significant effects ($P < 0.05$) as grey circles and non-significant effects ($P > 0.05$) as white circles.

zone, the allergen maxima occurred in the mid-autumn to winter period and minima occurred in mid-summer, with some variation over the 7 years. Allergen concentrations changed with children's bed type, but seasonal changes were not affected by the antimitic intervention.

The findings are generally consistent with seven shorter studies that showed bed Der p 1 peaks over the autumn (17–20, 22), summer (16) or summer–autumn period (7). This study showed that there was some variation in the annual timing of peaks, depending on the preceding seasonal climate. A recent European study showed that two unusually cold, successive winters shifted baseline

measurements of mite concentrations in beds, in that case affecting an intervention (36).

Data on the proliferation of mites in culture only provide a limited guide to interpreting observations in houses. Under stable culture conditions mite populations flourish between 25 and 30°C at humidity $> 60\%$ and drop precipitously at $< 60\%$. They also sharply decline under 20°C (37). All these conditions could be expected to occur in beds depending on occupancy and indoor microclimates. Population modelling is complicated by adaptive mechanisms to survive adverse conditions such as taking advantage of fluctuating humidity (38).

Our study shows the overall influence of humidity on allergen concentration was lagged by a period of 2 months (Fig. 3). This observation is similar to two other studies; one of which measured both allergen and mite numbers and showed allergen lagging mites by 2 months (16) and the other, measuring only allergen, showed allergen lagging humidity (20). While mite populations were not estimated in this study, the allergen peak in late autumn would be consistent with the accumulation of allergen following a peak in mite numbers during late summer when humidity was highest and average monthly temperatures peaked at more than 23°C (Fig. 1, regional relative humidity and temperature). This peak in mite numbers would have built up from the end of spring following the annual RH% minima, 6 months before the peak in allergen.

The two- to threefold differences in seasonal allergen seen in our studies are similar to that reported in several other studies (7, 17, 19, 20) but the explanation for Sydney may be different. Figure 1 shows concordance between indoor and outdoor climate measures, reflecting little use of centralized climate control in Sydney houses. Here, the absolute indoor humidity seldom ever falls below 7 g/m^3 and houses lack the severe dry winter 'heating season' of many parts of Europe and the USA which kills mites. In this year-round temperate climate, some times of the year are simply more favoured than others.

Although mite proliferation has been extensively studied, the factors that determine the production and loss of allergen from reservoirs are less well characterized. While the rate of feeding, and presumably allergen production, are strongly determined by humidity (5) the rate of biological breakdown of allergens at the high humidity found in beds, when microbial activity would also be greater, is not known.

The pattern of allergen fluctuations in the avoidance beds was similar to the non-intervention beds. This implies that either the interventions with both encasings and laundry did not suppress the climate-driven production of allergen by resident mites or that the allergens in these beds originated from other sites in the room and settled on the beds. We have previously shown that the intervention significantly reduced allergen (32), but as this study shows it had no effect on seasonal fluctuations amongst the three bed types analysed.

We found bassinets had lower concentration of mite allergen probably because many were purchased as new and many had linings that would have been frequently removed for laundry.

The lower peaks and concentrations during 1998 and 1999 early in the study, reflects the contributions from such beds. The high allergen concentrations observed during 1999 and mid-2001 may be explained by the fact that relative humidity remained higher than 55% during this period. The following gradual decline in allergen concentrations between mid-2001 and 2004 coincided with a local drought over this period, when annual rainfall in Sydney was 22% lower than the previous 3 years (39) and the annual relative humidity minima dropped below 55% (Fig. 1, regional relative humidity). This is analogous to macroclimate changes reported in the PIAMA study (36).

One confounder may be that changes in allergen may also be a function of changes in the type and quantity of bedding in use with different seasons. Although the intervention group was instructed to launder all bedding before introducing it, compliance with this instruction is not known. Some of the observed variability may also be attributable to participants changing their bed during the course of the study.

Seasonal variation in mite allergen concentrations in bedding may be relevant to seasonal variation in symptoms of allergic disease and in exacerbations of asthma. Seasonal changes in mite allergen have been demonstrated to accompany airway hyperresponsiveness in one study (21) and immunoglobulin E concentrations in two others (3, 40). We are currently further investigating the clinical correlations of these seasonal allergen fluctuations in Sydney.

Acknowledgments

We acknowledge the assistance of the Childhood Asthma Prevention Study research team. Research nurses included Nicola Vukasin, Craig Wainwright, Samantha Forbes, William Krause, Anne Tattam and Kitty Ng. We are especially grateful to the children and parents who made this study possible, whose commitment over several years enabled us to complete our work.

This study was supported by grants from National Health and Medical Research Council of Australia, Cooperative Research Centre for Asthma, New South Wales Department of Health and The Children's Hospital at Westmead, NSW, Australia. Catarina Almqvist was funded by the Swedish Heart Lung Foundation and the Swedish Society of Medicine.

References

1. Arlian LG, Morgan M, Neal J. Dust mite allergens: ecology and distribution. *Curr Allergy Asthma Rep* 2002;**2**:401–411.
2. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006;**368**:763–770.
3. Mimura T, Yamagami S, Amano S, Funatsu H, Arimoto A, Usui T et al. Allergens in Japanese patients with allergic conjunctivitis in autumn. *Eye* 2005;**19**:995–999.
4. Wahn U, von Mutius E. Childhood risk factors for atopy and the importance of early intervention. *J Allergy Clin Immunol* 2001;**107**:567–574.
5. Arlian LG. Humidity as a factor regulating feeding and water balance of the house dust mites *Dermatophagoides farinae* and *D. pteronyssinus* (Acari: Pyroglyphidae). *J Med Entomol* 1977;**14**:484–488.
6. Voorhorst R, Spieksma FT, Varekamp H. Biological aspects of the study of *D. pteronyssinus*. In: House-dust atopy and the house-dust mite *Dermatophagoides pteronyssinus*. Leiden: Staf-leu's Scientific Publishing Company, 1969:128–130.
7. Sidenius KE, Hallas TE, Poulsen LK, Mosbech H. A controlled intervention study concerning the effect of intended temperature rise on house dust mite load. *Ann Agric Environ Med* 2002;**9**:163–168.
8. Spieksma FT, Spieksma-Boezeman MI. The mite fauna of house dust with particular reference to the house-dust mite *Dermatophagoides pteronyssinus* (Trouessart, 1897) (Psoroptidae: Sarcoptiformes). *Acarologia* 1967;**9**:226–241.
9. Spieksma FTM, Zuidema P, Leupen MJ. High altitude and house-dust mites. *Br Med J* 1971;**1**:82–84.
10. Sharp JL, Haramoto FH. *Dermatophagoides pteronyssinus* (Trouessart) and other Acarina in house dust in Hawaii. *Proc Hawaii Entomol Soc* 1970;**20**:583–589.
11. Hughes AM, Maunsell K. A study of a population of house dust mite in its natural environment. *Clin Allergy* 1973;**3**:127–131.
12. Arlian LG, Bernstein IL, Gallagher JS. The prevalence of house dust mites, *Dermatophagoides* spp, and associated environmental conditions in homes in Ohio. *J Allergy Clin Immunol* 1982;**69**:527–532.
13. Arlian LG, Woodford PJ, Bernstein IL, Gallagher JS. Seasonal population structure of house dust mites, *Dermatophagoides* spp. (Acari: Pyroglyphidae). *J Med Entomol* 1983;**20**:99–102.
14. Domrow R. Seasonal variation in numbers of the house-dust mite in Brisbane. *Med J Aust* 1970;**2**:1248–1250.
15. Miyamoto J, Ouchi T. Ecological studies of house dust mites seasonal changes in mite populations in house dust in Japan. *Eisei Dobutsu* 1976;**27**:251–259.
16. Platts-Mills TAE, Hayden ML, Chapman MD, Wilkins SR. Seasonal variation in dust mite and grass-pollen allergens in dust from the houses of patients with asthma. *J Allergy Clin Immunol* 1987;**79**:781–791.
17. Chew GL, Higgins KM, Gold DR, Muilenberg ML, Burge HA. Monthly measurements of indoor allergens and the influence of housing type in a northeastern US city. *Allergy* 1999;**54**:1058–1066.

18. Garrett MH, Hooper BM, Hooper MA. Indoor environmental factors associated with house-dust-mite allergen (Der p 1) levels in south-eastern Australian houses. *Allergy* 1998;**53**:1060–1065.
19. Kalra S, Crank P, Hepworth J, Pickering CA, Woodcock AA. Absence of seasonal variation in concentrations of the house dust mite allergen Der p1 in south Manchester homes. *Thorax* 1992;**47**:928–931.
20. Miyazawa H, Sakaguchi M, Inouye S, Ikeda K, Honbo Y, Yasueda H et al. Seasonal changes in mite allergen (Der I and Der II) concentrations in Japanese homes. *Ann Allergy Asthma Immunol* 1996;**76**:170–174.
21. van der Heide S, De Monchy JG, De Vries K, Dubois AE, Kauffman HF. Seasonal differences in airway hyperresponsiveness in asthmatic patients: relationship with allergen exposure and sensitization to house dust mites. *Clin Exp Allergy* 1997;**27**:627–633.
22. van Strien RT, Koopman LP, Kerkhof M, Spithoven J, de Jongste JC, Gerritsen J et al. Mite and pet allergen levels in homes of children born to allergic and nonallergic parents: the PIAMA study. *Environ Health Perspect* 2002;**110**:A693–A698.
23. Munir AK, Einarsson R, Kjellman NI, Bjorksten B. Mite (Der p 1, Der f 1) and cat (Fel d 1) allergens in the homes of babies with a family history of allergy. *Allergy* 1993;**48**:158–163.
24. Lintner TJ, Brame KA. The effects of season, climate, and air-conditioning on the prevalence of *Dermatophagoides* mite allergens in household dust. *J Allergy Clin Immunol* 1993;**91**:862–867.
25. van der Heide S, de Monchy JG, de Vries K, Bruggink TM, Kauffman HF. Seasonal variation in airway hyperresponsiveness and natural exposure to house dust mite allergens in patients with asthma. *J Allergy Clin Immunol* 1994;**93**:470–475.
26. Chan-Yeung M, Becker A, Lam J, Dimich-Ward H, Ferguson A, Warren P et al. House dust mite allergen levels in two cities in Canada: effects of season, humidity, city and home characteristics. *Clin Exp Allergy* 1995;**25**:240–246.
27. Dharmage S, Bailey M, Raven J, Cheng A, Rolland J, Thien F et al. Residential characteristics influence Der p 1 levels in homes in Melbourne, Australia. *Clin Exp Allergy* 1999;**29**:461–469.
28. Murray AB, Ferguson AC, Morrison B. The seasonal variation of allergic respiratory symptoms induced by house dust mites. *Ann Allergy* 1980;**45**:347–350.
29. Zock J-P, Heinrich J, Jarvis D, Verlato G, Norback D, Plana E et al. Distribution and determinants of house dust mite allergens in Europe: The European Community Respiratory Health Survey II. *J Allergy Clin Immunol* 2006;**118**:682–690.
30. Marks GB, Tovey ER, Toelle BG, Wachinger S, Peat JK, Woolcock AJ. Mite allergen (Der p 1) concentration in houses and its relation to the presence and severity of asthma in a population of Sydney schoolchildren. *J Allergy Clin Immunol* 1995;**96**:441–448.
31. Habibullah M, Mahmic A, Jalaludin B, Tovey E, Mohsin M, Leeder SR. The effect of season on house dust mite allergen (Der P 1) concentrations in reservoir and aeroallergen samples in Australia. *Environ Health* 2004;**4**:35–44.
32. Marks GB, Mihrshahi S, Kemp AS, Tovey ER, Webb K, Almqvist C et al. Prevention of asthma during the first 5 years of life: a randomized controlled trial. *J Allergy Clin Immunol* 2006;**118**:53–61.
33. Mihrshahi S, Peat JK, Webb K, Tovey ER, Marks GB, Mellis CM et al. The childhood asthma prevention study (CAPS): design and research protocol of a randomized trial for the primary prevention of asthma. *Control Clin Trials* 2001;**22**:333–354.
34. Luczynska CM, Arruda LK, Platts-Mills TA, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, Der p I and Der f I. *J Immunol Methods* 1989;**118**:227–235.
35. Sharp JL, Haramoto FH. *Dermatophagoides pteronyssinus* (Trouessart) and other Acarina in house dust in Hawaii. *Proc Hawaii Entomol Soc* 1970;**20**:583–589.
36. Brunekreef B, van Strien R, Pronk A, Oldenwening M, de Jongste JC, Wijga A et al. La mano de DIOS...was the PIAMA intervention study intervened upon? *Allergy* 2005;**60**:1083–1086.
37. Crowther D, Wilkinson T, Biddulph P, Oreszczyn T, Pretlove S, Ridley I. A simple model for predicting the effect of hygrothermal conditions on populations of house dust mite *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *Exp Appl Acarol* 2006;**39**:127–148.
38. Pike AJ, Cunningham MJ, Lester PJ. Development of *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae) at constant and simultaneously fluctuating temperature and humidity conditions. *J Med Entomol* 2005;**42**:266–269.
39. Anonymous. Climate Data Online. The Australian Bureau of Meteorology. <http://www.bom.gov.au/climate/averages/>; accessed on 5 July 2007.
40. Arlian LG, Bernstein IL, Johnson CL, Gallagher JS. Ecology of house dust mites and dust allergy. In: Rodriguez JG, editor. Recent advances in acarology. Vol. II. New York: Academic Press, 1979:185–195.